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Research Article

# Three novel species and a new record of Pleosporales (Didymellaceae, Roussoellaceae) from China

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## Abstract

We are investigating saprobic Ascomycota in Guizhou Province, China. Fungal specimens collected from dead wood were identified based on morphological characteristics and multi-gene phylogenetic analyses of ITS, SSU, LSU, *β-tubulin*, *ef1-α*, and *rpb2* sequence data. Three novel species, *Neoroussoella guizhouensis*, *Roussoella guizhouensis*, and *Xenodidymella guizhouensis*, are introduced, along with one new geographical record, *Xenoroussoella triseptata*. This study contributes to our understanding of the diversity of Ascomycota in Guizhou Province and the classification in Roussoellaceae.

**Key words:** 3 new taxa, Ascomycota, diversity, morphology, *Neoroussoella*, *Roussoella*, *Xenodidymella*

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## Introduction

Pleosporales, the largest and most diverse order within the class Dothideomycetes, encompasses over 20,000 species across more than 220 families (Hyde et al. 2024a; Pem et al. 2024). Members of this order play key ecological and economic roles, including functioning as plant pathogens, saprobes, and endophytes on various host plants (Ariyawansa et al. 2020). Recent surveys indicated that Guizhou province is a hotspot for fungal biodiversity, particularly within its forested and bamboo-dominated ecosystems (Wijayawardene et al. 2021a; Sun et al. 2023; Yang et al. 2023; Zhang et al. 2023). With its subtropical climate, diverse topography, and high humidity, this region offers a fertile environment for fungal growth. Our study focuses on fungal specimens collected from decaying wood and bamboo culms in Guizhou province, China.

Roussoellaceae was defined with accommodating *Neoroussoella*, *Roussoella*, and *Roussoellopsis* (Liu et al. 2014). The family has been expanded to include more genera such as *Appendispora*, *Cytoplea*, *Elongatopedicellata*, *Immotthia*, and *Pararoussoella* (Bizzozero et al. 1885; Barr et al. 1987; Hyde et al. 1994; Ariyawansa et al. 2015; Jaklitsch et al. 2016; Wanasinghe et al. 2018; Pem et al. 2024). Although Jaklitsch et al. (2016) suggested that Roussoellaceae should be synonymous with

Thyridariaceae, this conclusion has not been universally accepted, and other studies supported Roussoellaceae as a distinct family (Liu et al. 2015; Tibpromma et al. 2017; Wijayawardene et al. 2017; 2018; Hyde et al. 2018). Currently, 13 genera are recognized within Roussoellaceae, based on comprehensive morphological and molecular data (Hongsanan 2020; Wijayawardene et al. 2021b; Li et al. 2023; Hyde et al. 2024a).

Didymellaceae is the largest and most diverse group in Pleosporales (Hyde et al. 2024b). This family comprises three main genera: *Ascochyta*, *Didymella*, and *Phoma*, and several allied phoma-like genera (Gruyter et al. 2009). Didymellaceae species are widely distributed across various hosts and habitats, with many species being important plant pathogens (Chen et al. 2015). Some species have quarantine significance, posing substantial challenges to regulatory organizations tasked with managing plant health and preventing the spread of plant diseases. Additionally, several species within Didymellaceae exhibit saprobic and endophytic lifestyles, colonizing a wide range of hosts (Aveskamp et al. 2008; Pem et al. 2024).

The present study introduced Roussoellaceae and Didymellaceae taxa isolated from dead wood in Guizhou province, supported by their distinct morphological and phylogenetic characteristics. We use a polyphasic approach as guidelines for introducing new species (Maharachchikumbura et al. 2021), and this comprehensive analysis enhances our understanding of fungal diversity in the region and contributes valuable insights into their taxonomy and ecological roles.

## Materials and methods

### Sample collection and fungal isolation

Dead wood samples were collected in Guiyang, Guizhou province, China, in September 2023. The specimens were taken to the laboratory in envelopes to observe and check their morphological characteristics. The samples were observed by a SMZ-T4 continuous zoom stereomicroscope (Optec stereomicroscope SMZ-T4, China), and the fruiting bodies on the samples were picked with needles and then made into slides to observe their morphological characteristics under the Zeiss microscope (Axioscope 5, China). Pure cultures were obtained by single spore isolation as described in Senanayake et al. (2020). After 2–3 days of spore germination, it was transferred to potato dextrose agar medium (PDA) for pure culture at 25 °C for 14 days. A Nikon digital camera (Canon 700D) was used to photograph the fruiting bodies on natural substrates. Zeiss Scope5 compound microscope Axioscope 5 (Carl Zeiss Microscopy GmbH, Jena, Germany) DIC and an axial camera were used to take photographs of microstructure. We measured 50 *conidia* and 10 *conidiogenous cells*. Dried holotype specimens were preserved in the herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University (HGUP). Ex-type cultures were deposited in the Culture Collection at the Department of Plant Pathology, Agriculture College, Guizhou University, P.R. China (GUCC). Taxonomic information of the new species was submitted to MycoBank (www.mycobank.org), and accession numbers were provided in the taxonomy section of this paper.

## DNA extraction, amplification via PCR, and sequencing

Fungal DNA was extracted from the fresh mycelium grown on the PDA (c = 40.1 g/l) medium in an incubator at 25 °C for 30–40 days. Fresh mycelium was scraped using a sharpened plastic straw and placed into a 1.5 mL centrifuge tube. Then, it was ground into powder with a grinder. Genomic DNA was extracted following the instruction book of the Biospin Fungus Genomic DNA Extraction Kit (BioFlux®). The DNA was stored at −20 °C for long-term usage. Information on primers used for the amplification of internal transcribed spacers (ITS), small subunit rDNA (SSU), large subunit rDNA (LSU), translation elongation factor 1-α gene region (*ef1-α*), RNA polymerase II second largest subunit (*rpb2*), and beta-tubulin (*β-tubulin*) genes is presented in Table 1. The amplification reactions were performed by following the protocol: 20 μL of PCR reaction mixture contained 1 μL of DNA template, 1 μL each of the forward and reverse primers (10 μm), 7 μL of double-distilled water ($ddH_2O$), and 10 μL of 2Mix (Vazyme Biotech Co., Ltd). The PCR thermal cycling program conditions are presented in Table 1. PCR products were sequenced using Sanger sequencing conducted by Sangon Biotech (Shanghai) Co., Ltd. The PCR products were sequenced, and the sequence data were deposited in GenBank, as shown in Table 1 (Dai et al. 2017).

## Phylogenetic analyses

Phylogenetic analysis was performed using DNA sequence data obtained from GenBank (https://www.ncbi.nlm.nih.gov/, accessed on 21 September 2024) (Table 2) and following previous publications (Dai et al. 2022; Wu et al. 2023). Sequence data were aligned using the online MAFFT v. 7.307 (http://mafft.cbrc.jp/alignment/server/, accessed on 21 September 2024; Katoh et al. 2019), and trim ambiguously aligned positions were done using trimAl (21 September 2024; Capella-Gutiérrez et al. 2009). The software BioEdit version 7.2.5 was utilized to make additional manual edits (Hall 1999). Ambiguous regions were excluded from the analysis, and gaps were treated as missing data. The aligned sequences that will be used for maximum likelihood (ML) were concatenated

**Table 1.** The primers and polymerase chain reaction (PCR) thermal cycling programs for each locus.

| Genes | Primers | PCR Thermal Cycle Protocols |
|---|---|---|
| ITS | ITS4 | (94 °C, 30 s, 55 °C, 50 s, 72 °C, 1 min) 35 cycles |
| | ITS5 | |
| LSU | LR0R | |
| | LR5 | |
| SSU | NS1 | |
| | NS4 | |
| *ef1-α* | EF1-983F | |
| | EF1-2218R | |
| *rpb2* | fRPB2-5f | (94 °C, 1 min, 52 °C, 2 min, 72 °C, 90 s) 40 cycles |
| | fRPB2-7cr | |
| *β-tubulin* | Btub2Fd | (94 °C, 30 s, 58 °C, 30 s, 72 °C, 90 s) 35 cycles |
| | Btub4Rd | |

**Table 2.** Species and GenBank accession numbers of DNA sequences used in the phylogenetic analysis.

| Taxon | Strain* | Host | Origin | GenBank accession numbers | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | ITS | LSU | *rpb2* | *ef1-α* | *β-tubulin* |
| *Elongatopedicellata lignicola* | MFLUCC 15-0642 | dead wood | Thailand | KX421369 | KX421368 | - | - | - |
| *Neoroussoella alishanense* | FU31016 T | dead culms | China | MK503816 | MK503822 | MN037756 | MK336181 | - |
| *N. bambusae* | MFLUCC 11-0124 T | bamboo | Thailand | KJ474827 | KJ474839 | KJ474856 | KJ474848 | - |
| *N. chiangmaiensis* | MFLUCC 22-0168 T | dead culms | Thailand | NR189405 | OQ065735 | OQ186450 | OQ186448 | - |
| *N. clematidis* | MFLU 17-1467 T | *Clematis subumbellata* | Thailand | NR170813 | - | - | - | - |
| | MFLUCC 17-2061 | *Clematis subumbellata* | Thailand | MT310632 | MT214587 | MT394701 | MT394645 | - |
| *N. entadae* | MFLUCC 18-0243 | *Leucaena* sp. | Thailand | MK347786 | MK348004 | MK434866 | MK360065 | - |
| | NI213 | *Magnolia* sp. | Thailand | OL703580 | OL457703 | | OM505027 | |
| *N. fulvicomae* | MFLU 17-1471 T | *Clematis fulvicoma* | Thailand | NR170814 | - | MT394702 | MT394646 | - |
| *N. heveae* | MFLUCC 17-2069 | *Clematis subumbellata* | Thailand | MT310634 | MT214589 | MT394703 | MT394647 | - |
| *N. lenispora* | GZCC 16-0020 T | dead branch | China | NG068779 | - | - | - | - |
| *N. leucaenae* | MFLUCC 17-0927 T | *Pterocarpus* sp. | Thailand | NR165226 | - | MK434896 | MK360066 | - |
| | MFLUCC 18-1544 | *Leucaena* sp. | Thailand | MK347767 | MK347984 | MK434876 | MK360067 | - |
| *N. magnoliae* | MFLU 18-1022 | *Magnolia grandiflora* | China | MK801232 | MK801230 | | MK834373 | |
| ***N. guizhouensis*** | **GUCC 24-0196** | **dead wood** | **China** | **PQ404885** | **PQ475844** | **PQ438559** | **PQ438562** | **-** |
| | **GUCC 24-0197 T** | **dead wood** | **China** | **PQ404886** | **PQ475842** | **PQ438557** | **PQ438560** | **-** |
| | **GUCC 24-0198** | **dead wood** | **China** | **PQ404887** | **PQ475843** | **PQ438558** | **PQ438561** | **-** |
| *N. lignicola* | MUT 5373 T | *Padina pavonica* | Italy | NR169908 | MN556321 | MN605916 | MN605896 | - |
| *N. peltophora* | MFLUCC 21-0071 T | *Peltophorum dubium* | Thailand | NG149003 | MZ567206 | MZ605442 | MZ605441 | - |
| *N. sedimenticola* | CGMCC 3 22468 | *Sediment* | China | OQ798948 | OQ758143 | OQ809007 | OQ809045 | - |
| *N. solani* | CPC 26331 T | *Solanum mauritianum* | France | NR145198 | - | - | - | - |
| *N. thailandica* | NI258 T | *Magnolia* sp. | Thailand | OL703581 | OL457704 | ON502386 | OM505028 | - |
| *Nothoroussoella irregularis* | CGMCC 3.22466 | sediment | China | NR191243 | OQ758150 | OQ809014 | OQ809052 | - |
| | CGMCC 3.22467 | sediment | China | - | OQ758151 | OQ809015 | OQ809053 | - |
| *Pseudoroussoella chromolaenae* | MFLUCC 17-1492 T | Dead aerial stems | Thailand | NR168861 | MT214439 | - | MT235769 | - |
| | MFLUCC 17-2062 | *Clematis subumbellata* | Thailand | MT310635 | MT214590 | MT394704 | MT394648 | - |
| *P. elaeicola* | MFLUCC 17-2059 | *Clematis subumbellata* | Thailand | - | MT214591 | MT394705 | - | - |
| | MFLUCC:17-1483 | *Chromolaena odorata* | Thailand | MT214348 | MT214442 | MT235808 | MT235772 | - |
| *Roussoella angusta* | MFLUCC 15-0186 T | *bamboo* | Thailand | - | KT281979 | - | - | - |
| *R. aquatica* | MFLUCC 18-1040 T | submerged wood | China | NR171975 | NG073797 | - | - | - |
| *R. chiangraina* | MFLUCC 10-0556 T | bamboo | Thailand | KJ474828 | KJ474840 | KJ474857 | KJ474849 | - |
| *R. doimaesalongensis* | MFLUCC 14-0584 T | bamboo | Thailand | KY026584 | KY000659 | KY678394 | KY651249 | - |
| *R. guttulata* | MFLUCC 20-0102 T | bamboo | Thailand | NR172428 | NG075383 | MW022187 | MW022188 | - |
| ***R. guizhouensis*** | **GUCC 24-0199 T** | **bamboo** | **China** | **PQ404882** | **PQ475845** | **PQ399769** | **PQ438563** | **-** |
| | **GUCC 24-0200** | **bamboo** | **China** | **PQ404883** | **PQ475847** | **PQ399770** | **PQ438564** | **-** |
| | **GUCC 24-0201** | **bamboo** | **China** | **PQ404884** | **PQ475846** | **PQ399771** | **PQ438565** | **-** |
| *R. hysterioides* | CBS 546.94 | - | France | KF443405 | KF443381 | KF443392 | KF443399 | - |
| *R. intermedia* | NBRC 106245 | *Sasa kurilensis* | Japan | KJ474831 | AB524624 | - | - | - |
| *R. japanensis* | MAFF 239636 T | *Sasa veitchii* | Japan | KJ474829 | AB524621 | AB539101 | AB539114 | - |
| | GMBCC1117 | *bamboo* | China | OM891811 | OM884023 | ON098382 | ON098345 | - |
| *R. kunmingensis* | KUMCC 18-0128 T | *bamboo* | China | MH453491 | MH453487 | MH453484 | MH453480 | - |
| | GMBCC1057 | *bamboo* | China | OM891798 | OM884014 | ON098362 | ON098354 | - |

| Taxon | Strain* | Host | Origin | GenBank accession numbers | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | ITS | LSU | *rpb2* | *ef1-α* | *β-tubulin* |
| *R. magnatum* | MFLUCC 15-0185 T | *bamboo* | China | - | KT281980 | - | - | - |
| *R. margidorensis* | MUT 5329 T | *Padina pavonica* | Italy | KU314944 | MN556322 | MN605917 | MN605897 | - |
| *R. mediterranea* | MUT 5369 T | *Padina pavonica* | Italy | KU314947 | MN556324 | MN605919 | MN605899 | - |
| *R. multiloculate* | GMBCC1056 T | bamboo | China | OM891799 | OM884015 | ON098369 | ON098343 | - |
| | GMBCC1071 | bamboo | China | ON159383 | OM755586 | ON098368 | ON098342 | - |
| *R. neopustulans* | MFLUCC 11-0609 T | bamboo | Thailand | KJ474833 | KJ474841 | - | KJ474850 | - |
| | MFLUCC 12-0003 | bamboo | Thailand | KU940130 | KU863119 | - | - | - |
| *R. nitidula* | MFLUCC 11-0182 T | *Bambusa* | Thailand | KJ474835 | KJ474843 | KJ474859 | KJ474852 | - |
| | MFLUCC 11-0634 | bamboo | Thailand | KJ474834 | KJ474842 | KJ474858 | KJ474851 | - |
| *R. padinae* | MUT 5503 T | *Padina pavonica* | Italy | KU158170 | MN556327 | MN605922 | MN605902 | - |
| | GMBCC1126 | bamboo | China | OM891816 | OM884025 | ON098383 | ON098356 | - |
| | GMBCC1121 T | bamboo | China | OM891814 | OM755608 | ON098378 | ON098346 | - |
| | IFRDCC 3103 | bamboo | China | ON228188 | ON228184 | ON244450 | ON244452 | - |
| *R. pseudohysterioides* | MFLUCC 13-0852 T | bamboo | Thailand | KU940131 | KU863120 | - | KU940198 | - |
| | KUMCC 18-0111 | bamboo | China | MH453490 | MH453486 | MH453483 | MH453479 | - |
| *R. pustulans* | MAFF 239637 T | *Sasa kurilensis* | Japan | KJ474830 | AB524623 | AB539103 | AB539116 | - |
| *R. scabrispora* | MFLUCC 11-0624 T | bamboo | Thailand | KJ474836 | KJ474844 | KJ474860 | KJ474853 | - |
| *R. scabrispora* | GMBCC1104 | bamboo | China | OM891807 | OM755616 | ON098373 | ON098349 | - |
| *R. siamensis* | MFLUCC 11-0149 T | Bambusa | Thailand | KJ474837 | KJ474845 | KJ474861 | KJ474854 | - |
| | GMBCC1119 T | bamboo | China | OM891813 | OM884024 | ON098379 | ON098357 | - |
| | IFRDCC 3101 | bamboo | China | ON228187 | ON228183 | ON244451 | ON244453 | - |
| *R. thailandica* | MFLUCC 11-0621 T | bamboo | Thailand | KJ474838 | KJ474846 | - | - | - |
| *R. tuberculata* | MFLUCC 13-0854 T | bamboo | Thailand | KU940132 | KU863121 | - | KU940199 | - |
| | GMBCC1123 | bamboo | China | OM891815 | OM755613 | ON098380 | ON098352 | - |
| *R. uniloculata* | GMBCC1110 T | bamboo | China | OM891809 | OM801286 | ON098374 | ON098360 | - |
| | DDQ01005-2 | bamboo | China | OM891817 | OM884026 | ON098375 | ON098361 | - |
| *R. verrucispora* | CBS 125434 T | *Sasa kurilensis* | Japan | KJ474832 | AB524622 | AB539102 | AB539115 | - |
| *R. yunnanensis* | KUMCC 18-0115 T | bamboo | China | MH453492 | MH453488 | - | MH453481 | - |
| *Roussoellopsis macrospora* | MFLUCC 12-0005 T | bamboo | Thailand | KJ739604 | KJ474847 | KJ474862 | KJ474855 | - |
| *Ro. tosaensis* | KT 1659 | bamboo | Japan | - | AB524625 | AB539104 | AB539117 | - |
| *Setoarthopyrenia chromolaenae* | MFLUCC 17-1444 T | *Chromolaena odorata* | Thailand | NG070153 | MT214438 | MT235805 | MT235768 | - |
| *Xenoroussoella triseptata* | MFLUCC 17-1438 T | *Chromolaena odorata* | Thailand | MT214343 | MT214437 | MT235804 | MT235767 | - |
| | KNUF-20-NI009 | Soil | South Korea | LC719282 | LC719283 | LC723532 | LC723531 | - |
| | **GUCC 24-0202** | **dead wood** | **China** | **PQ404879** | **PQ475839** | **PQ399766** | **PQ438566** | - |
| | **GUCC 24-0203** | **dead wood** | **China** | **PQ404880** | **PQ475840** | **PQ399767** | **PQ438567** | **-** |
| | **GUCC 24-0204** | **dead wood** | **China** | **PQ404881** | **PQ475841** | **PQ399768** | **PQ438568** | **-** |
| *Versicolorisporium triseptatum* | JCM 14775 T | *Pleioblastus chino* | Japan | AB365596 | AB330081 | - | - | - |
| | NMX1222 | Bamboo | China | OL741378 | OL741318 | - | - | - |
| *Xenodidymella applanata* | CBS 205.63 | *Rubus idaeus* | Netherlands | GU237798 | - | KP330402 | - | GU237556 |
| | CBS 195.36 T | *Rubus idaeus* | Netherlands | KT389548 | - | MT018280 | - | KT389852 |
| *Xenodid. asphodeli* | CBS 375.62 T | *Asphodelus albus* | France | KT389549 | - | KT389689 | - | MT005716 |
| | CBS 499.72 | *Asphodelus ramosus* | Italy | KT389550 | - | MT018282 | - | KT389853 |
| *Xenod. camporesii* | MFLUCC 17-2309 T | *Dipsacus* sp. | Italy | NR169976 | - | - | - | MN871955 |
| *Xenod. catariae* | CBS 102635 | *Nepeta catenaria* | Netherlands | GU237727 | - | KP330404 | - | GU237524 |
| *Xenodid. clematidis* | MFLUCC 16-1365 T | *Clematis vitalba* | Italy | MT310600 | - | - | - | - |
| *Xenodid. glycyrrhizicola* | IRAN 4162C;, SCUA-Ahm-S45 | *Ziziphus mauritiana* | Iran | MZ145256 | - | MZ169396 | - | MZ169403 |
| *Xenod. glycyrrhizicola* | CBS 684.97T | *Glycyrrhiza lepidota* | New Zealand | MN973606 | - | MT018283 | - | MT005717 |

| Taxon | Strain* | Host | Origin | GenBank accession numbers | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | ITS | LSU | *rpb2* | *ef1-α* | *β-tubulin* |
| ***Xenod. guizhouensis*** | **GUCC 24-0205 T** | **dead wood** | **China** | **PQ404876** | **-** | **PQ399763** | **-** | **PQ399760** |
| | **GUCC 24-0206** | **dead wood** | **China** | **PQ404877** | **-** | **PQ399764** | **-** | **PQ399761** |
| | **GUCC 24-0207** | **dead wood** | **China** | **PQ404878** | **-** | **PQ399765** | **-** | **PQ399762** |
| *Xenod. humicola* | CBS 220.85 | *Franseria* sp. | U.S.A. | GU237800 | - | KP330422 | - | GU237617 |
| *Xenod. iranica* | SCUA-Ahm-Sh1-2 | *Callistemon citrinus* | Iran | MZ145253 | - | - | - | MZ169400 |
| | IRAN 4142CT;, SCUA-Saf-Sh1 | *Callistemon citrinus* | Iran | MZ145252 | - | - | - | MZ169399 |
| *Xenod. menthae* | SCUA-Ahm-W4 | *Mentha* × *piperita* | Iran | OK257018 | - | OK247739 | - | OK247745 |
| | SCUA-Ah-W4-2 | *Mentha* × *piperita* | Iran | OK257019 | - | OK247740 | - | OK247746 |
| *Xenod. saxea* | CBS 419.92T | Corroded mediterranean marble | Germany | GU237860 | - | KP330429 | - | GU237655 |
| *Xenodidymella* sp. | CBS 141233 | *Anchusa italica* | Iran | MN973608 | - | MT018285 | - | MT005719 |
| *Xenod. weymaniae* | CBS 144960 T | - | Netherlands | MN823588 | - | MN824613 | - | MN824762 |
| *Neod. xanthina* | CBS:383.68 T | *Delphinium* sp. | Netherlands | NR135994 | - | KP330431 | - | GU237668 |
| | CBS:168.70 | *Delphinium* sp. | Netherlands | KT389533 | - | MT018290 | - | KT389831 |
| *Didymella subrosea* | CBS 733.79 T | *Abies alba* | France | NR170787 | - | MT018174 | - | MT005643 |
| *D. subglobispora* | CBS 364.91 | *Ananas sativus* | - | MN973531 | - | MT018153 | - | MT005634 |

*T = Type. The strains in this study are in bold. CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands; CPC: Culture collection of Pedro Crous, housed at the Westerdijk Institute; GUCC: Herbarium of the Department of Plant Pathology, Agricultural College, Guizhou University, China; MFLUCC: Mae Fah Luang University Culture Collection, Thailand.

using Sequence Matrix v. 1.7.8 (Vaidya et al. 2011) and conducted with IQ-TREE (Nguyen et al. 2015; Trifinopoulos et al. 2016) via the IQ-TREE web server (accessed on 21 September 2024, http://iqtree.cibiv.univie.ac.at). The concatenated sequence alignments used for Bayesian inference analysis (BI) were acquired from MEGA versions 7.0.14 and 10.1.0, as reported by Kumar et al. (2016) and Tamura et al. (2021), respectively. Geneious Prime® 2023.2.1 Java Version 11.0.18+10 (64-bit) software (Biomatters Inc., Boston, USA) was used to convert the file format. Two approaches were applied to determine the phylogenetic relationships of the newly identified strains: maximum likelihood (ML) and Bayesian inference (BI) analyses. The final phylogenetic topologies were visualized with FigTree v.1.4.0 (http://tree.bio.ed.ac.uk/software/figtree/) and were modified in Microsoft Office PowerPoint 2021 (Microsoft, Redmond, USA).

## Results

### Phylogenetic analyses

The phylogenetic analyses (Fig. 1) utilized a comprehensive dataset comprising four loci (ITS, LSU, *rpb2*, and *ef1-α*), including 79 strains of Roussoellaceae species, with two outgroup strains, *Versicolorisporium triseptatum* (strain JCM 14775) and *V. triseptatum* (strain NMX1222). The final alignment comprised 3,026 characters (ITS: 1–425, LSU: 426–1210, *rpb2*: 1211–2233, and *ef1-α*: 2234–3026), including gaps. Both maximum likelihood (ML) and Bayesian inference (BI) methods were conducted. The ML tree was selected to represent the phylogenetic relationships of Roussoellaceae taxa. The analyses revealed that the newly isolated strains (GUCC 24-0197, GUCC 24-0196, and GUCC 24-0198) formed a distinct branch with strong statistical support (MLBS 81%/BIPP < 0.80). This clade emerged as a sister group to *Neoroussoella leucaenae* (MFLUCC 18-1544), supported by

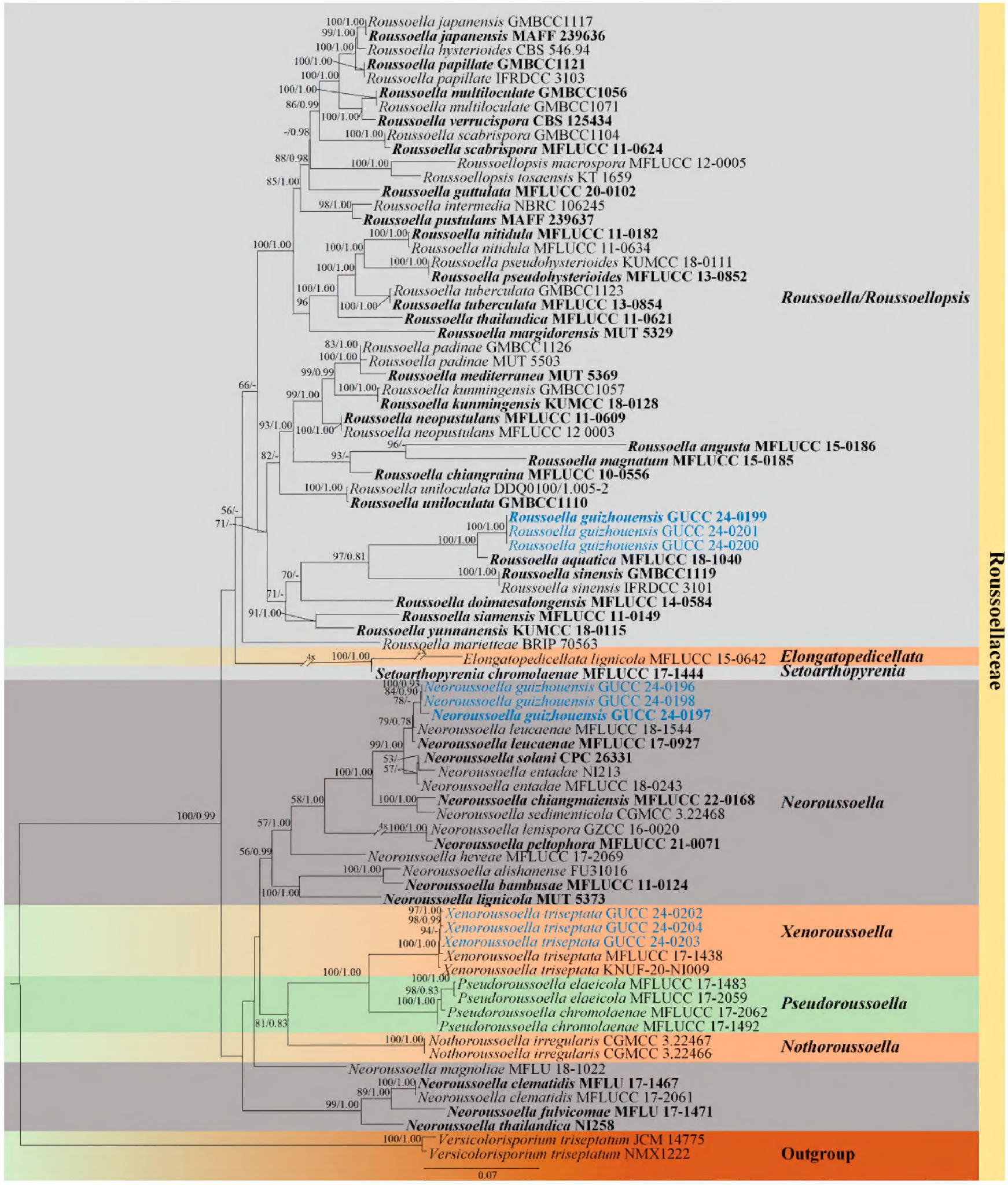


**Figure 1.** Phylogenetic tree generated from maximum likelihood analysis based on combined ITS, LSU, *rpb2*, and *ef1-α* sequence data for 79 strains of Roussoellaceae. This tree is rooted with two strains of *Versicolorisporium triseptatum* (JCM 14775 and NMX1222). Maximum likelihood bootstrap values ≥ 50% and Bayesian posterior probabilities ≥ 0.80 (MLBS/BIPP) are given at the nodes. The species obtained in this study are blue, and the ex-type taxa are bold. Bar = 0.07 represents the estimated number of nucleotide site substitutions per branch. The second phylogenetic tree (Fig. 2) was constructed based on a comprehensive polygenic analysis of the ITS, *rpb2*, and *β-tubulin* gene regions, utilizing a dataset that included 22 strains of *Xenodidymella*, with two outgroup strains, *Didymella subrosea* (CBS 733.79) and *D. subglobispora* (CBS 364.91). The final alignment, which accounted for gaps, comprised 1,412 characters (ITS: 1–484, *rpb2*: 485–1079, *β-tubulin*: 1080–1412). These datasets were then used to perform maximum likelihood (ML) and Bayesian inference (BI) analyses. The ML tree was the primary representation for illustrating the phylogenetic relationships among the diverse taxa within *Xenodidymella*. This tree elucidated the evolutionary positioning and interrelationships of *Xenodidymella* and its related species. Notably, our strains (GUCC 24-0205, GUCC 24-0206, and GUCC 24-0207) formed an obviously distinct lineage, separate from other *Xenodidymella* species, with high support by 100% MLB and 1.00 BIPP.

moderate statistical values (78% MLBS/0.69 BIPP). Similarly, strains GUCC 24-0199, GUCC 24-0200, and GUCC 24-0201 formed a distinct branch, with robust support (100% MLBS and 1.00 BIPP). This clade was positioned as a sister group to *R. aquatica* (MFLUCC 18-1040), with exhibited high support (100% MLBS/1.00 BIPP). The strains GUCC 24-0202, GUCC 24-0203, and GUCC 24-0204 clustered with two strains of *Xenoroussoella triseptata* (MFLUCC 17-1438 and KNUF-20-NI009), which demonstrated 100% MLBS and 1.00 BIPP support.

## Taxonomy

### *Neoroussoella* Jian K. Liu, Phook. & K.D. Hyde, *Phytotaxa* 181(1): 21 (2014)

*Neoroussoella* (Roussoellaceae) was introduced by Liu et al. (2014) to accommodate species that exhibited morphological characteristics distinct from other genera. *Neoroussoella* spp. are typically saprobic, inhabiting decaying wood and plant material, and have been found primarily in subtropical and tropical regions. Morphologically, the genus is characterized by immersed to semi-immersed ascomata, cylindrical to clavate asci, and hyaline, septate ascospores (Pem et al. 2024). Phylogenetic analyses based on multilocus DNA sequencing have further supported the placement of *Neoroussoella* as a distinct genus within Roussoellaceae.

### *Neoroussoella guizhouensis* S.Q. Guo, K.D. Hyde & Yong Wang bis, sp. nov.

MycoBank No: 856167

Fig. 3

**Etymology.** The name refers to Guizhou Province, where the fungus was collected.

**Type.** China • Guizhou Province, Guiyang City, 26°6'N, 106°7'E, 1071 m, 7 September 2023, from dead wood culms, coll. S.Q. Guo, HGUP 24-0071 (holotype), ex-type culture GUCC 24-0197 (ITS: PQ404886, LSU: PP949847, *rpb2*: PQ438557, *ef1-α*: PQ438560).

**Description.** ***Saprobic*** on dead wood culms. ***Sexual morph*:** undetermined. ***Asexual morph*:** On the natural substrate: ***Pycnidia*** 81–137 × 65–1198 µm ($\bar{x}$ = 108.5 × 81.9 µm, n = 10), ellipsoidal to nearly circular, inlaid on the surface of the substrate with a black ring at the edge, transparent in the center, and smooth in surface texture. On PDA: ***Conidiophores*** reduced to ***conidiogenous cells*. *Conidiogenous cell*** 9–35 × 1–6 µm ($\bar{x}$ = 20 × 3.8 µm, n = 10), enteroblastic, indeterminate, discrete, unbranched, clavate or ampulliform to lageniform, hyaline, aseptate, smooth-walled. ***Conidia*** (2.9–)3.4–3.5(–4) × (2–)2.4–2.5(–2.6) µm ($\bar{x}$ = 3.5 × 2.5 µm, n = 50), oblong to ellipsoid, with rounded to obtuse ends, hyaline, aseptate, smooth-walled.

**Culture characteristics.** Under dark conditions at 25 °C, the colony diameter can reach 80 mm within seven days on PDA medium. Center of the colony is light gray, accompanied by a white ring. Hyphae at the edge are sparse, slightly irregular, and lace-like, and several vertical stripes spread out at the center. Reverse side is black to light red, and the edge is yellowish.

**Material examined.** China • Guizhou Province, Guiyang City, 26°6'N, 106°7'E, 1071 m, 7 September 2023, from dead wood culms, coll. J.E. Sun, HGUP 24-0071 (***holotype***), living culture GUCC 24-0197, GUCC 24-0196, and GUCC 24-0198.

**Notes.** *Neoroussoella guizhouensis* is a saprobic fungus found on dead wood culms. According to the multi-gene phylogenetic analysis (Fig. 1), our isolates formed a distinct clade, sister to *Neoroussoella leucaenae*, which was previously reported from decaying pods of *Leucaena* sp. and *Pterocarpus* sp. in Thailand (Jayasiri et al. 2019). This relationship is supported by statistical values of 79% MLBS and 0.78 BIPP (Fig. 1). On PDA, colonies of *N. guizhouensis* display a light gray center surrounded by a white ring, distinguishing it from *N. solani*, which exhibits white to lavender-gray colonies and produces rosy buff to grayish-rose pigments (Mochizuki et al. 2017). Morphological data for *N. leucaenae* and *N. entadae* on PDA are unavailable in the literature. However, on MEA, *N. leucaenae* shows gray to brown hyphal growth with a radially arranged brown edge and a two-layered reverse side, dark brown and brown. In contrast, *N. entadae* displays irregular edges with a thin, light-colored outer layer embedded in the medium, a three-layered reverse side of dark brown with distinct boundaries, and scattered pycnidia throughout the culture medium (Jayasiri et al. 2019). Phylogenetically, the new species was grouped with *N. guizhouensis*, *N. leucaenae*, *N. solani*, and *N. entadae*. However, the morphological distinctions are evident among those species, particularly in the conidiogenous cells. Although all species exhibit bottle-shaped sporogenic structures, *N. leucaenae* is characterized by flask- to cylindrical-shaped cells with a thicker neck and nearly round base. In contrast, *N. guizhouensis* has a narrow-necked bottle shape, whereas *N. solani* shows a broader base, with some cells appearing nearly oval. *N. entadae* displays phialidic, ampulliform to cylindrical conidiogenous cells. The dimensions of the conidiogenous cells differ significantly among the species: *N. guizhouensis* (9–35 × 1–6 µm), *N. leucaenae* (5.5–9 × 3–4 µm), *N. solani* (5–10 × 3.5–5 µm), and *N. entadae* (3.5–5.6 × 0.7–1.8 µm). Conidia size comparisons also highlight differences: *N. guizhouensis* (2.9–4 × 2–2.6 µm), *N. leucaenae* (3.5–4.5 × 1.9–2.6 µm), *N. solani* (3.9–5.3 × 1.9–2.2 µm), and *N. entadae* (3–4 × 1.7–1.9 µm). While *N. guizhouensis* and *N. leucaenae* are similar in spore size, they have shorter lengths and narrower widths compared to *N. solani* and exhibit similar lengths but narrower widths compared to *N. entadae*. Additionally, nucleotide sequence comparisons of the *ef1-α* and *rpb2* loci revealed differences between *N. guizhouensis* (GUCC 24-0197) and *N. leucaenae* (MFLUCC 17-0927), with 2-bp and 16-bp discrepancies, respectively. Based on these findings, *N. guizhouensis* is established as a new species.

### *Roussoella* Sacc., Atti Inst. Veneto Sci. lett., ed Arti, Sér. 6 6: 410 (1888)

*Roussoella* was introduced by Saccardo and Paoletti (1888) and subsequently designated as the type genus of Roussoellaceae by Liu et al. (2014). *Roussoella* species are predominantly saprobic, commonly found on terrestrial or aquatic flowering plants and on submerged wood in freshwater habitats, with a particular affinity for bamboo and palm trees (Dong et al. 2020). Morphologically, the sexual state of *Roussoella* is characterized by immersed apothecia, cylindrical asci, and spindle-shaped ascospores (Hyde et al. 2013; Liu et al. 2014; Dai et al. 2022), while the asexual state is distinguished by ellipsoidal or ampulliform conidia (Dai et al. 2017; Jiang et al. 2019; Dong et al. 2020).

***Roussoella guizhouensis*** **S. Q. Guo, K.D. Hyde & Yong Wang bis, sp. nov.**
MycoBank No: 856168
Fig. 4

**Etymology.** The name refers to Guizhou Province, where the fungus was collected.

**Type.** China • Guizhou Province, Guiyang City, 26°6'N, 106°7'E, 1071 m, 24 September 2023, from dead bamboo culms, coll. J.F. Mo, HGUP 24-0072 (holotype), ex-type culture GUCC 24-0199 (ITS: PQ404882, LSU: PP949847, *rpb2*: PQ399769, *ef1-α*: PQ438563).

**Description.** ***Saprobic*** on dead bamboo culms. ***Sexual morph*****:** undetermined. ***Asexual morph*****: *Pycnidia*** 998–3121 × 143–853 µm ($\bar{x}$ = 1749.6 × 430.9 µm, n = 10), stromatic on the substrate, develops above the epidermis and becomes raised when mature, ellipsoidal to irregular in shape, with a rough surface ranging in color from reddish-brown to black, composed of numerous dense, glossy black ***stromata*** globules stacked layer upon layer. ***Stroma*** ranges in color from light brown to black, being transparent and shaped like a hemisphere to a near-perfect sphere, with uniform size, smooth surface, glossy, with half embedded in the natural substrate and the other half exposed, a circular aperture is present at the top. On PDA medium: ***Pycnidia*** superficial, float above the ***stromata***, shaped like hemispheres or spheres, appearing as viscous, mucoid masses with a flat base, colour ranging from dark brown to black, lacking a pycnidial wall, release brownish-yellow ***conidia*** mass. ***Conidiophores*** reduced to ***conidiogenous cells***. ***Conidiogenous cell*** 5–8 × 1–2 µm ($\bar{x}$ = 6.2 × 1.7 µm, n = 10), enteroblastic, indeterminate, discrete, unbranched, clavate or ampulliform to lageniform, transparent to light brown, aseptate, smooth-walled. ***Conidia*** (1.7–)2.2–2.3(–2.6) × (1.3–)1.5–1.6(–1.9) µm ($\bar{x}$ = 2.3 × 1.6 µm, n = 50), oblong to ellipsoid, with rounded to obtuse ends, light brown to transparent, smooth-walled.

**Culture characteristics.** Under dark conditions at 25 °C, the colony reaches a diameter of 90 mm within seven days on PDA medium. Colony is dense, exhibiting a rough surface and a regular shape with a fluffy margin. Center is velvety, characterized by a grayish-white coloration surrounded by gray margins. Reverse side is grayish-black, featuring a small grayish-white circle in the middle, which produces a black pigment.

**Material examined.** China • Guizhou Province, Guiyang City, 26°6'N, 106°7'E, 1071 m, 24 September 2023, from dead bamboo culms, coll. J.F. Mo, HGUP 24-0072 (***holotype***), living culture GUCC 24-0199, GUCC 24-0200 and GUCC 24-0201.

**Notes.** Based on the multigene phylogenetic tree (Fig. 1), our strains grouped into a distinct clade, forming a sister clade to *Roussoella aquatica* (MFLUCC 18-1040, Dong et al. 2020), found in submerged wood from a stream in Yunnan, China, with 100 MLBS/BIPP 1.00 statistical support. A comparison of nucleotide data within ITS and LSU loci revealed discrepancies between *R. guizhouensis* and *R. aquatica* (ITS: 16 bp and LSU: 7 bp). Morphologically, *R. guizhouensis* is distinguishable from *R. aquatica* by having larger conidiogenous cells (5–8 × 1–2 µm *vs*. 3–4 × 1.5–2 µm) and smaller conidia (1.8–2.7 × 1.3–1.9 µm *vs*. 2.7–3.5 × 2–2.5 µm). Thus, we introduce *Roussoella guizhouensis* as a new taxon.

***Xenodidymella*** **Qian Chen & L. Cai, Stud. Mycol. 82: 205 (2015)**

*Xenodidymella* was established based on the type species, *X. applanata*, along with three additional species: *X. asphodeli*, *X. catariae*, and *X. humicola*. Phylogenetic analyses based on multilocus data have demonstrated that these species formed a monophyletic lineage within phoma-like taxa (Chen et al. 2015). Most species of *Xenodidymella* are recognized as plant pathogens, capable of causing various diseases in crops and plants worldwide (Boerema et al. 2004). Additionally, several *Xenodidymella* species function as saprobes, inhabiting living and dead plant tissues (Hyde et al. 2020; Phukhamsakda et al. 2020).

***Xenodidymella guizhouensis*** **S.Q. Guo, K.D. Hyde & Yong Wang bis, sp. nov.**
MycoBank No: 856169
Fig. 5

**Etymology.** The name refers to Guizhou Province, where the fungus was collected.

**Type.** China • Guizhou Province, Guiyang City, 26°6'N, 106°6'E, 1071 m, 7 September 2023, from dead wood culms, coll. S.M. Fu, HGUP 24-0073 (holotype), ex-type culture GUCC 24-0205 (ITS: PQ404876, *rpb2*: PQ399763, *β-tubulin*: PQ399760).

**Description.** ***Saprobic*** on dead wood culms. ***Sexual morph*****:** undetermined. ***Asexual morph*****:** ***Stromata*** grow above the epidermis and become hemispherically raised when mature. ***Stromata*** 61–587 μm high, 67–489 μm wide, fattened at the base, those ***stromata*** distributed singly, in groups, or scattered, and their color ranges from dark brown to black. On PDA medium, grows on epidermis and bulges when matures. ***Conidiophores*** degenerated into sporogenous cells. ***Conidiogenous cells*** 8–17 × 1–5 μm ($\bar{x}$ = 17.9 × 2.6 μm, n = 10) wide, phialidic, ampulliform, or lageniform, hyaline, smooth-walled, and have two types of spore-producing structures: one type, long, thin tentacles that extend irregularly outward, other type, short and wide, and twisted together to form knots, and the ends of both rounded. ***Conidia*** (1.7–)2.7–2.8(–3) × (0.7–)0.9–1(–1.5) μm ($\bar{x}$ = 2.5 × 1 μm, n = 50) oblong, hyaline, aseptate, with two small polar guttules at both ends, smooth, thin-walled.

**Culture characteristics.** ***Colonies*** on PDA reached a diameter of 20 mm after seven days at 25 °C. Hyphae white at the center, radiating outward from a pale-yellow inner ring to a light yellowish-brown periphery with a milky white outer ring. The colony reverse exhibited a dark yellow to bright yellow pigmentation.

Colonies on OA reached 32 mm after seven days at 25 °C. Initially, it was a small circle formed by uniform and sparse white hyphae. After 14 days, it grew into slightly sparse hyphae with white clutter inside and dense white hyphae at the edge in a slightly regular circle. On the surface, hyphae balls with uneven sizes from orange to white and tiny black spots were scattered, and sporogenous structures could be observed.

**Material examined.** China • Guizhou Province, Guiyang City, 26°6'N, 106°6'E, 1071 m, 7 September 2023, from dead wood culms, coll. S.M. Fu, HGUP 24-0073 (holotype), living culture GUCC 24-0205, GUCC 24-0206, and GUCC 24-0207.

**Notes.** Phylogenetic analyses (Fig. 2) revealed that the three isolates (GUCC 24-0205, GUCC 24-0206, and GUCC 24-0207) formed an independent branch.

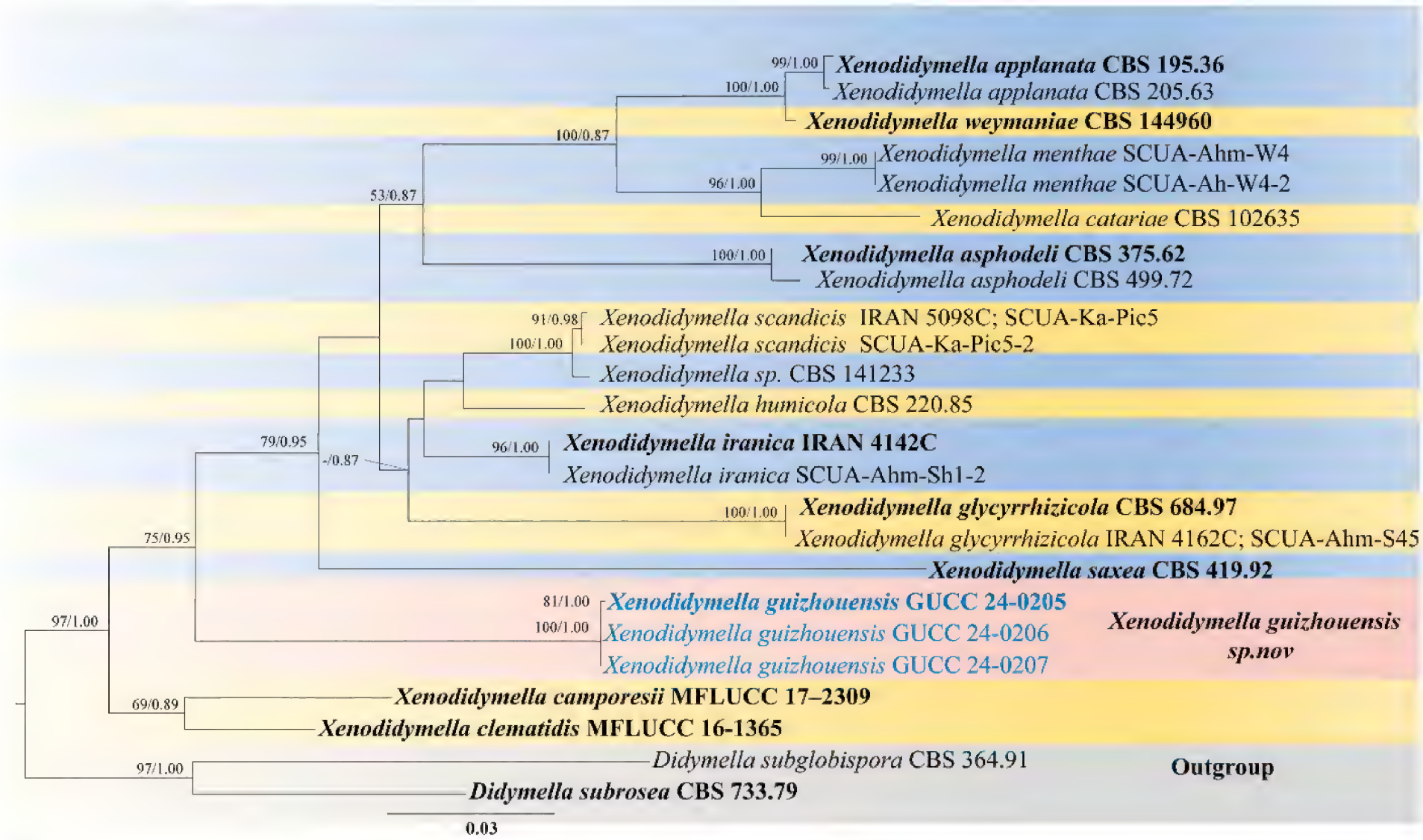


**Figure 2.** The phylogenetic tree generated from maximum likelihood analysis based on combined ITS, *β-tubulin*, and *rpb2* sequence data for 22 strains of *Xenodidymella*. This tree is rooted with *Didymella subrosea* (CBS 733.79) and *D. subglobispora* (CBS 364.91). Maximum likelihood bootstrap values ≥ 50% and Bayesian posterior probabilities ≥ 0.80 (MLBS/BIPP) are given at the nodes. The species obtained in this study are blue, and the ex-type taxa are bold. Bar: 0.03 represents the estimated number of nucleotide site substitutions per branch.

In morphology, they can be distinguished from *Xenod. clematidis* by their smaller conidiogenous cells (10–17(–20) × 1–2 µm *vs*. 2–6(–12) × 2.3–3.3 µm), although the conidiogenous cell size for *Xenod. saxea* remains undetermined. Additionally, *Xenod. guizhouensis* produces smaller *conidia* than both *Xenod. clematidis* and *Xenod. saxea* (2.9–4.3 × 1.7–2.4 µm *vs*. 4–8 × 2–5 µm and 3.5–7.5 × 2.5–4 µm), respectively.

### *Xenoroussoella* Mapook & K.D. Hyde, Fungal Diversity 101: 93 (2020)

*Xenoroussoella* is a genus within Roussoellaceae, classified under Pleosporales (Mapook et al. 2020). This genus comprises a group of fungi notable for their ecological roles as saprobes and plant pathogens. Recent phylogenetic studies have further elucidated the relationships within this genus, demonstrating its distinct placement within Roussoellaceae and highlighting its diversity (Mapook et al. 2020; Ryu et al. 2022).

### *Xenoroussoella triseptata* Mapook & K.D. Hyde, Fungal Diversity 101: 93 (2020)

Fig. 6

**Description.** ***Saprobic*** on dead wood culms. ***Sexual morph*:** see Mapook et al. (2020). ***Asexual morph*: *Pycnidia*** on natural host, stromatic, developing above the epidermis and becoming raised when mature. ***Stromata*** 60–786 ×

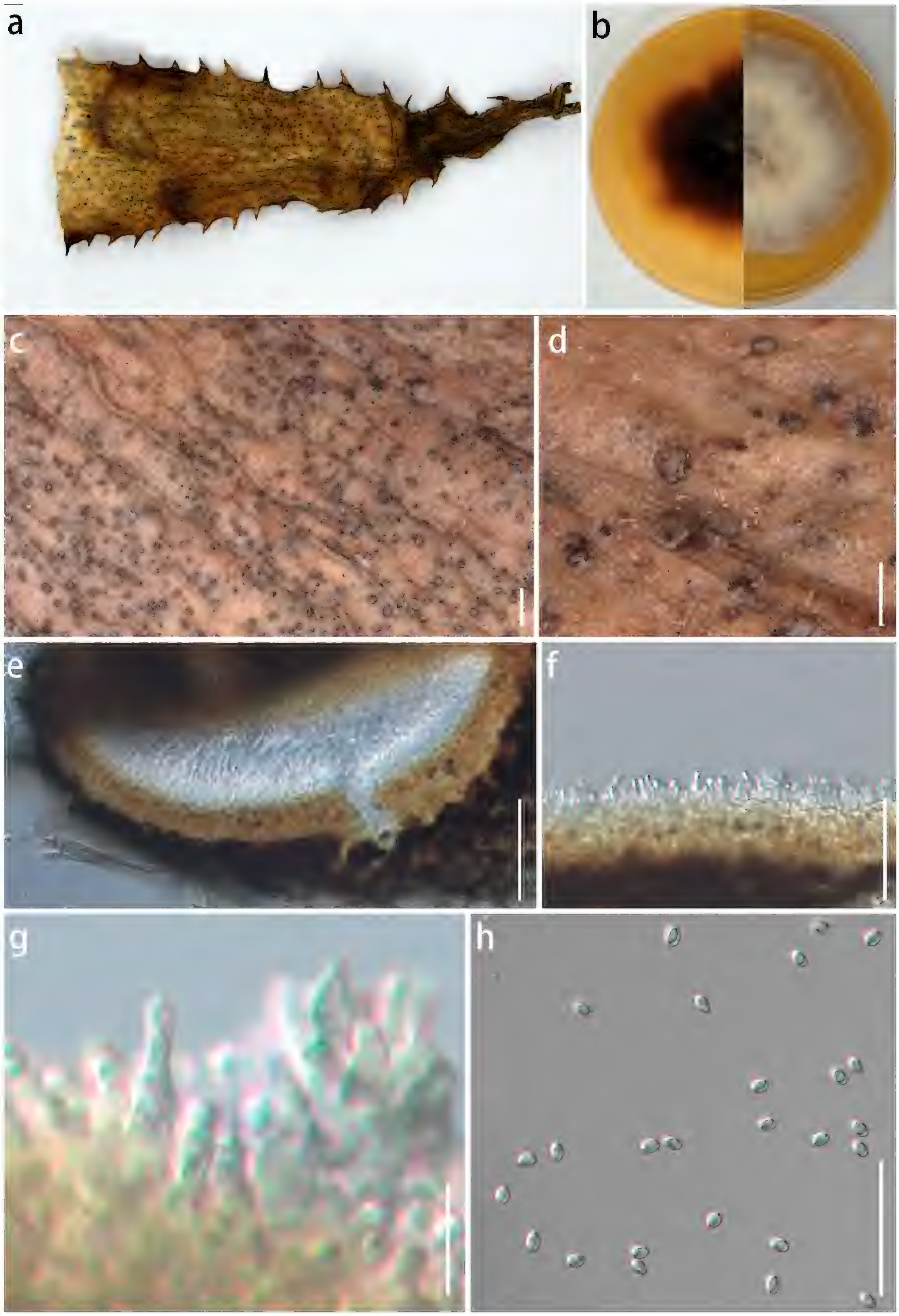


**Figure 3.** *Neoroussoella guizhouensis* (GUCC 24-0197, holotype) **a** appearance on host surface **b** cultures on PDA from above and below **c, d** black ascostromata on host surface **e–g** conidiophores, conidiogenous cells, and conidia (h) conidia. Scale bars: 1000 µm (**c**); 250 µm (**d**); 50 µm (**e, f**); 12.5 µm (**g**); 25 µm (**h**).

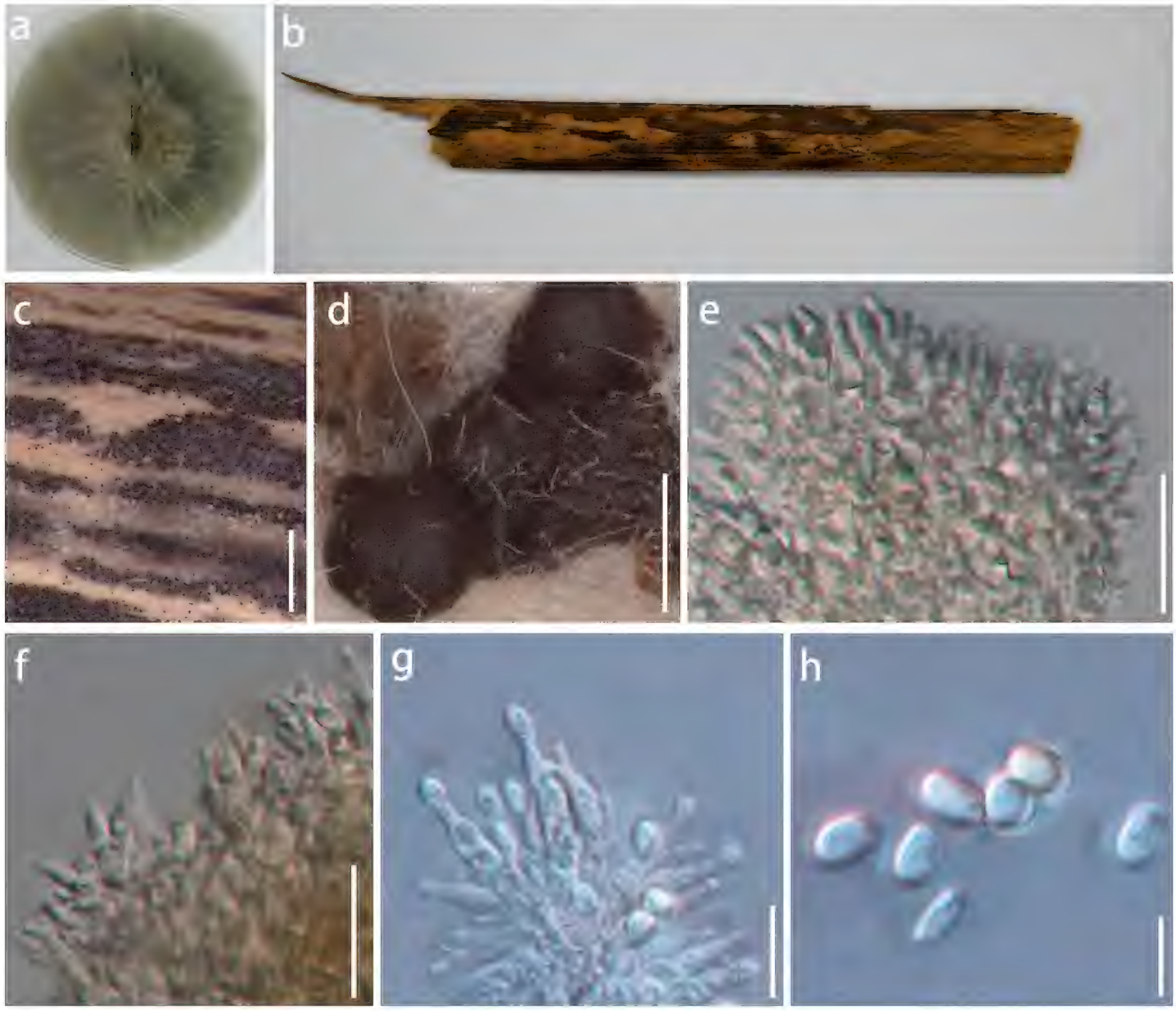


**Figure 4.** *Roussoella guizhouensis* (GUCC 24-0199, holotype) **a** cultures on PDA from above and below **b** bamboo specimen **c, d** black conidiostromata on host surface **e–g** conidiophores, conidiogenous cells, and conidia **h** conidia. Scale bars: 500 μm (**c**); 200 μm (**d**); 25 μm (**e, f**); 15 μm (**g**); 2.5 μm (**h**).

43–306 μm ($\bar{x}$ = 320.8 × 172.0 μm, n = 10), with a color ranging from dark brown to black, rough surface with tiny protrusions, some exhibit a certain degree of gloss. and exhibit circular, oval, or irregular shapes, adhering tightly to the surface of decaying wood. On PDA medium, ***Conidiophores*** reduced to ***conidiogenous cells***. ***Conidiogenous cell*** 8–17 × 2–5 μm ($\bar{x}$ = 11.5 × 3 μm, n = 10), enteroblastic, indeterminate, discrete, unbranched, clavate or ampulliform, aseptate, hyaline, smooth-walled. ***Conidia*** (3.3–) 3.7–3.8 (–4.7) × (2.8–) 3.1–3.2 (3.6) μm ($\bar{x}$ = 3.8 × 3.2 μm, n = 50), oblong to ellipsoid or rounded to obtuse ends, aseptate, hyaline to pale yellow, smooth-walled.

**Culture characteristics.** Under dark conditions at 25 °C, the colony reaches a diameter of 15 mm within seven days on PDA medium. The colony is dense, smooth, and irregularly shaped, with a lace-like margin and compact structure. The center is grayish-white, bordered by a white lace-like margin, while the outer edge is grayish-black. The reverse side of the colony is black, producing a dark pigment.

**Distribution.** China, Korea, Thailand.

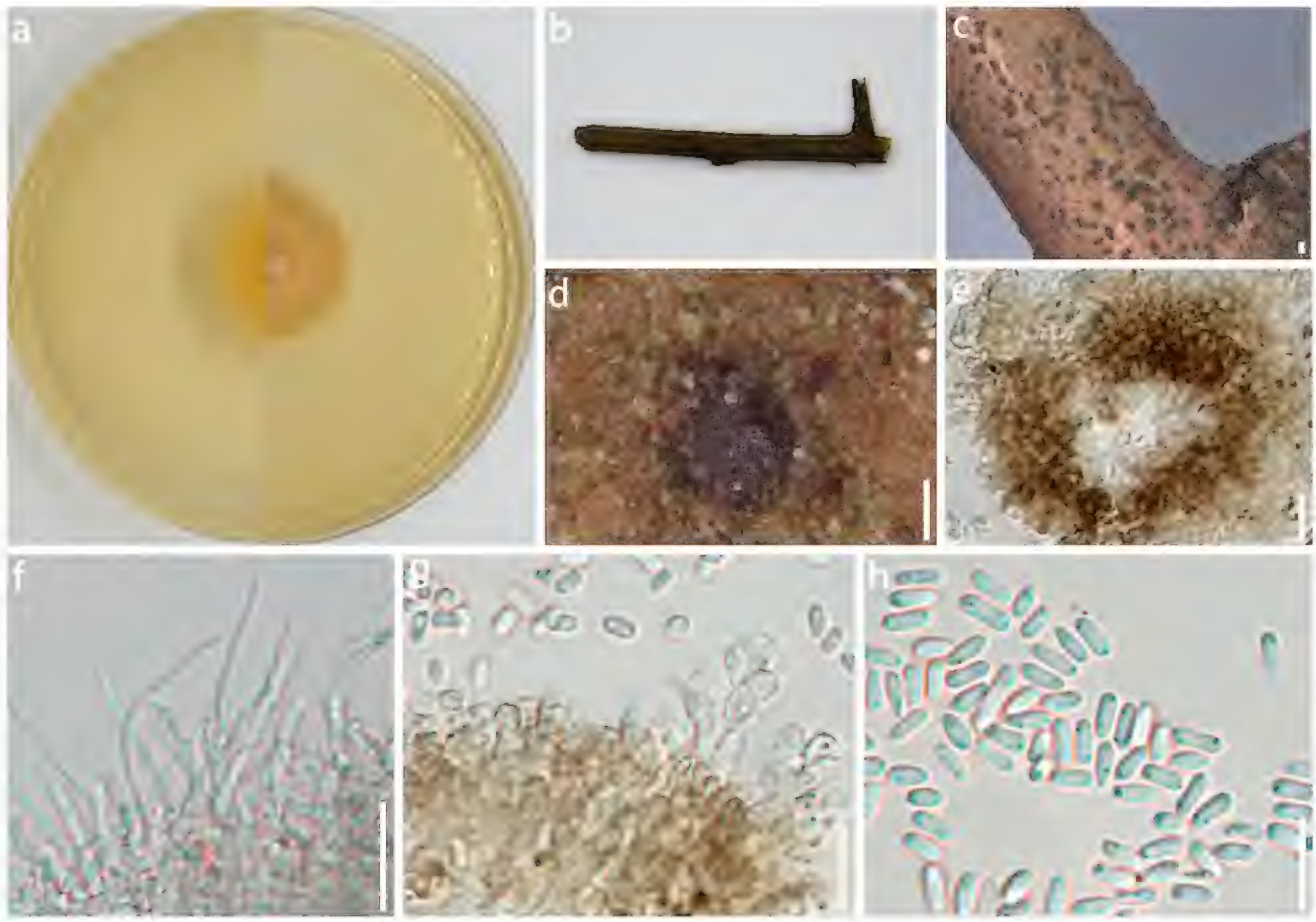


**Figure 5.** *Xenodidymella guizhouensis* (GUCC 24-0205, holotype) **a** cultures on PDA from above and below **b** appearance on host surface **c, d** black *ascostromata* on host surface **e–h** *conidiophores*, *conidiogenous cells*, *conidia*. Scale bars: 500 µm (**c**); 100 µm (**d**); 2.5 µm (**e**); 12.5 µm (**f**); 5 µm (**g**); 2.5 µm (**h**).

**Material examined.** CHINA • Guizhou Province, Guiyang City, 26°6'N, 106°73'E, 1071 m, 7 September 2023, from dead wood culms, coll. J.E. Sun, HGUP 24-0074 (holotype), ex-type: GUCC 24-0202 (ITS: PQ404879, LSU: PQ475839, *rpb2*: PQ399766, *ef1-α*: PQ438566), living culture: GUCC 24-0203 and GUCC 24-0204.

**Notes.** Phylogenetic analyses (Fig. 1) revealed that the newly isolated strains (GUCC 24-0202, GUCC 24-0203, and GUCC 24-0204) clustered closely with two strains of *Xenoroussoella triseptata* (MFLUCC 17-1438 and KNUF-20-NI009). This species was initially reported from dead stems of *Chromolaena odorata* in Thailand, with its sexual morph documented by Mapook et al. (2020). Subsequently, Ryu et al. (2022) described an asexual morph of this species isolated from soil in Korea. In the current study, sequence data from the ITS, LSU, *ef1-α*, and *rpb2* loci of newly isolated strains were identical to those of *X. triseptata*, confirming their conspecific status. The morphological characteristics of the asexual morph of the new strains were consistent with those previously described by Ryu et al. (2022) based on observations from the PDA medium. Conidiogenous cells were ampulliform to doliiform, producing unicellular conidia. Conidia were globose to ellipsoid with a blunt apex containing one or two guttules. The *conidia* contained one or two guttules, measuring 2.7–5.1 × 1.6–3.3 µm (Ryu et al. 2022). Thus, GUCC 24-0202, GUCC 24-0203, and GUCC 24-0204 were identified as *Xenoroussoella triseptata*, marking the first recorded occurrence in China.

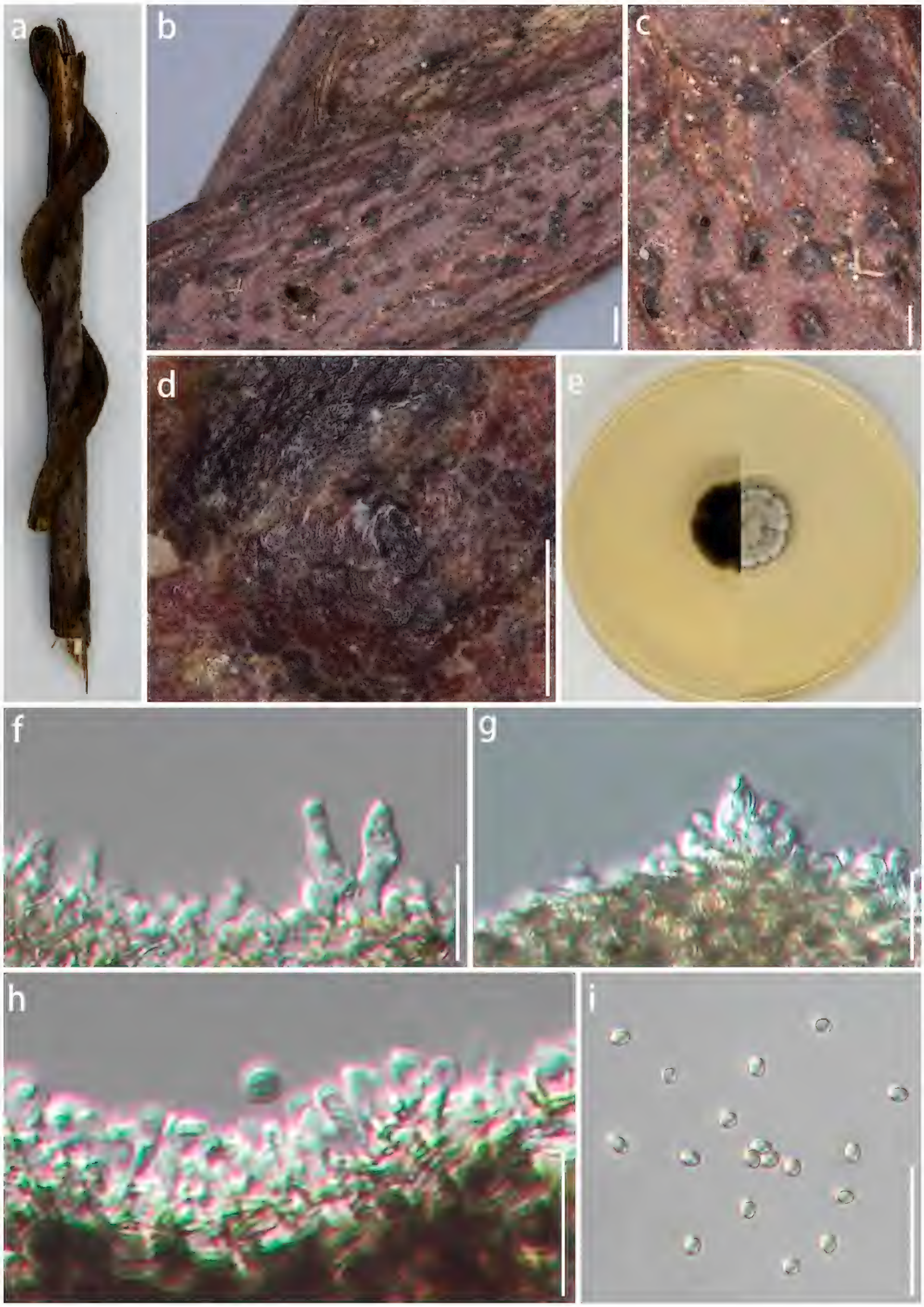


**Figure 6.** *Xenoroussoella triseptata* (GUCC 24-0202, new geographical record) **a** appearance on host surface **e** cultures on PDA from above and below **b, c, d** black ascostromata on host surface **f–h** conidiophores, conidiogenous cells, and conidia (i) conidia. Scale bars: 500 µm (**b**); 250 µm (**c**); 100 µm (**d**); 12.5 µm (**f–h**); 25 µm (**i**).

## Discussion

Recent extensive surveys across various ecosystems in Guizhou have documented a rich abundance of both saprobic and symbiotic fungi, which play critical ecological roles in nutrient cycling through the decomposition of organic matter (Chuzho et al. 2017; Duan et al. 2022; Luo et al. 2022; Sun et al. 2022, 2023; Wang et al. 2022; Zou et al. 2022; Cai and Zhao 2023; Hyde et al. 2023; Dong et al. 2024; Hu et al. 2024; Wei et al. 2024a, 2024b). Notably, researchers have identified novel and previously unrecorded species, enhancing our understanding of fungal diversity in the province (Dissanayake et al. 2024; Sui et al. 2024; Tian et al. 2024). Our study focused on two families, Didymellaceae and Roussoellaceae, underscoring the significance of Guizhou Province as a hotspot for fungal biodiversity (Wijayawardene et al. 2021a).

Here we introduce *Neoroussoella guizhouensis* and *Xenodidymella guizhouensis* based on morphology and multi-gene analyses. *Roussoella* is a speciose genus with 74 species (Yu et al. 2024). During this study we collected three strains of *Roussoella guizhouensis*, confirming the assumption of Bhunjun et al. (2022) that more species are likely to be discovered in species-rich genera. Furthermore, *Xenoroussoella triseptata*, previously documented in Korea and Thailand, were reported in China for the first time. In addition, based on the single-gene phylogenetic relationships of ITS, LSU, *rpb2*, and *ef1-α* sequences, we found that in the Roussoellaceae, the single-gene tree of *rpb2* was the most informative gene for determining the phylogenetic position of taxa within these genera. This shows that adding protein-coding genes to the analysis greatly facilitates species-level identification.

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## Additional information

### Conflict of interest

The authors have declared that no competing interests exist.

### Ethical statement

No ethical statement was reported.

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## Author contributions

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## Data availability

All of the data that support the findings of this study are available in the main text.

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